PURIFICATION PROCESS FOR MANUFACTURING A HIGH PURE ACARBOSE

Background of the Invention

1.Filed of the Invention

The present invention relates to a process for manufacturing high pure acarbose, more particularity, and to a process which uses alcohol for precipitation and separation, a strongly cation exchange chromatography and an immobilized enzyme affinity chromatography for manufacturing a high pure acarbose to treat diabetes.

2. Description of the Related Art

Acarbose, O-4,6-Dideoxy-4-[[[1S-(1 α ,4 α ,5 α ,6 α)]-4,5,6,-trihydroxy-3-(hydroxymethyl)-2-cyclohexe

n-1-yl]amino]- α -D-glucopyranosyl-(1 → 4)-O- α -D-glucopyranosyl-(1 → 4)-D-glucose , $C_{25}H_{43}NO_{18}$, Mw 645.63 , it is an oligo-derivatives. The acarbose inhibits the activity of α -glucosidase at the edge in small intestine by invertibility for slowly turning carbohydrates complex and disaccharide into glucose absorbed by human to decrease concentration of triglycerol and insulin in blood and blood sugar.

In early 1970, the acarbose could improve the ratio of meat and fat, so it uses to add in the feed to feed the animals such as pig. Recently, the researches find the acarbose could control the blood sugar of NIDDM and decreases the insulin value after diet for preventing diabetic cardiovascular complication, but it can not directly change the insulin resistance. The acarbose only has a few aftereffects, such as

abdominal distension, borborygmus and diarrhea, being away after treating a period, and it hardly affects the health. The glucobay of the Bayer is approved firstling in 1995 by FDA. So far, the manufacturing acarbose is mainly used *Actinoplanes sp.* or *Streptomyces glaucescens*.

The DOH of Taiwan adjusts the adjusting blood sugar material to be health food. Further, the fat reason of the Easterner is eating polysaccharide different from the westerner is eating fat. Therefore, the acarbose not only treats diabetes, but also uses in the diet food.

US. Pat. 4,062,950 of recover and purification process for manufacturing acarbose discloses that acarbose-containing fermentation broth is discolored by anion resins or activated carbons in the acid

condition, and acarbose are absorbed by activated carbons in the neutral condition and are eluted by ethyl alcohol solution or acetone solution in the acid condition. The elute passes through the cation exchange chromatography, acarbose are finally washed by the acid or base solution. The eluted liquid is counteracted and concentrated in the vacuum, and the 85% purity of the acarbose is precipitated by the organic solvent. The high purity of the acarbose can be manufactured if the exchange chromatography uses celluloses to be a matrix. Further, the liquid is concentrated and precipitated by the organic solvent to get a high purity of the acarbose. The process is complicate because the process must use the activated carbons for absorbing and the exchange chromatography process many times for purification

of acarbose.

US. Pat. 4,174,439 mixes cation and anion exchange resin into acarbose-containing fermentation broth to absorb acarbose and elutes the acarbose by deionized water. The acarbose solution process twice cation and anion exchange resin and is eluted by hydrochloric, and process neutralizing treatment by an anion exchange resin and frozen dry to get 52%~58% purity of the acarbose.

Further, US. Pat. 4,666,776 and US. Pat. 4,767,850 improve US. Pat. 4,174,439 to use strongly cation exchange resin and be washed by hydrochloric, and process neutralizing treatment by an anion exchange resin and frozen dry to get 79%~82% purity of the acarbose.

The above mentions of purifying the acarbose

process all repeat the anion and cation exchange chromatography to get the acarbose solution and finally use cation exchange chromatography to get a high concentration acarbose. But, the purity of acarbose is hard to be a medical drug.

US. Pat. 4,904,769 discloses which taking impure acarbose passes through weakly cation exchange chromatography containing carbonyl, cellulose, dextran in specific temperature and pH value to get 90% purity acarbose. The process is complicated and uses weakly ion exchange chromatography being an expansive process resulting in high manufacturing cost.

And, WO. 99/07720 discloses which taking an impure acarbose manufacturing by US. Pat. 4,174,439, US. Pat. 4,666,776 and US. Pat. 4,767,850 passes

through a strongly cation exchange chromatography containing non-aromatic to get high pure acarbose, and the processes typically has complicated process and high manufacturing cost.

Summary of the Invention

According to above mention, the present invention improves the complicated process and high manufacturing cost, and get a high pure acarbose being a medical drug.

The present invention considers processes and material of above mentions to improve an impure acarbose manufacturing process which applies the solubility between the acarbose and alcohol or methyl alcohol and absorbs the acarbose by strongly exchange resin and eliminates like acarbose by sodium chloride and ammonia solution. The 75%~80% purity acarbose could

get by eluting high concentration ammonia solution, and finally passes through α-glucosidase column to get up 95% purity acarbose to overcome the high manufacturing cost and complicated processes of prior art.

Brief Description of the Drawings

The accompanying drawing is included to provide a further understanding of the invention, and is incorporated in and constitutes a part of this specification. The drawing illustrates an embodiment of the invention and, together with the description, serves to explain the principles of the invention. In the drawing,

Fig. 1 is a flow chart showing a purification process for manufacturing a high pure acarbose of the present invention;

Fig. 2 is flow chart showing a purification process for manufacturing a high pure acarbose of Example 1 of the present invention;

Fig. 3 is flow chart showing a purification process for manufacturing a high pure acarbose of Example 2 of the present invention;

Fig. 4 is flow chart showing a purification process for manufacturing a high pure acarbose of Example 3 of the present invention; and

Fig. 5 is flow chart showing a purification process for manufacturing a high pure acarbose of Example 4 of the present invention.

Detailed Description of the preferred Embodiments

Refer to Fig. 1, the present invention discloses a purification process for manufacturing high pure

acarbose comprises the steps of: step 10, start; step 15, adding alcohol in acarbose-containing fermentation broth for precipitation; step 20, passing sediments through strongly cation exchange resin and processing an immobilized enzyme affinity chromatography process. The present invention discloses a purification and purifying acarbose process from acarbose containing fermentation broth to get a high pure acarbose to treat diabetes. The strongly cation exchange chromatography uses styrene divinylbenzene copolymer without methoxymethylmethacrylamide to be a resin matrix, and the enzyme of the immobilized enzyme affinity chromatography uses α -amyloglucosidase(α -glucoamylase).

Further, an upper liquid of the

acarbose-containing fermentation broth is made by centrifugal effect or filter and concentrates 1/10 volume by a rotary evaporators concentrating system. Then, adding adequate ethyl alcohol solution or methyl alcohol solution takes an upper liquid by centrifugating, and the upper liquid concentrates to consistency. Finally, the consistency uses ethyl alcohol to get a sediment of the acarbose, and the sediment is solved by distilled water to be 200 mg/mL concentration and adjusts pH=5~9 to be a mixing liquid.

The process of ion exchange resin describes using strongly cation exchange resin, AMBERJET 1200 H resin or AMBERJET 1200 Na(Rohm and Hass Company), is washed by deionized water till pH value of an upper liquid is large than 4. Then, taking strong

cation exchange resin containing 20-200 mg sugar/mL adds into the mixing liquid blending 10~30 minutes, and taking a part of resin is washed several times by distilled water. The resin is washed by NaCl to get a lot of acarbose-like sugars, and is eluted by 0.75N ammonia solution. Finally, the resin is solved by 1.5N ammonia solution to get acarbose, and concentrating the acarbose and using ethyl alcohol to get a precipitation which the purity of acarbose is 75~80%.

The impure acarbose powders add adequate distilled water to adjust the pH value between five and nine, and passes through a column containing AMBERJET 4400 OH resin and α-amyloglucosidase. Firstly, the column is washed one to four times volume as column and solves 55~75℃ distilled water. Then, collecting the acarbose concentrates and uses

the ethyl alcohol to get sediment. The sediment is cooled and dried to get the purity up 95% acarbose.

Example 1:

Refer to Fig. 2, the present invention comprises the steps of:

Step 100, eliminating myselium from acarbose-containing fermentation broth by centrifugating or filtering;

Step 102, concentrating filtrate or an upper liquid 1000 ml of the acarbose-containing fermentation broth to be consistency by a concenteration system;

Step 104, adding adequate ethyl alcohol to the consistency and blending to be a solution;

Step 106, taking an upper liquid from the solution after blending 30 minutes by centrifugating;

Step 108, concentrating the upper liquid to be a

consistency by the concentrating system;

Step 110, taking the consistency into 99.9% ethyl alcohol, which the amount of ethyl alcohol is nice times volume as the consistency, to get a consistency liquid;

Step 112, taking sediment from the consistency liquid by centrifugating and solving the sediment by water to get an impure acarbose solution;

Step 114, getting the impure acarbose solution being 10% purity and being 1560 mg by HPLC;

Step 116, blending a strongly cation exchange resin, AMBERJET 1200 H resin (Rohm and Hass Company), with the acarbose solution 10 minutes to get a resin;

Step 118, using 1.0N sodium chloride solution to eliminate an impurity in the resin;

Step 120, using 0.75N ammonia solution to eliminate a rest of the impurity in the resin;

Step 122, eluting the resin with 1.5N ammonia solution to get a high pure acarbose, which the purity of acarbose is 60%, 1220 mg.

Example 2:

Refer to Fig. 3, the present invention comprises the steps of:

Step 200, adjusting pH value between six and seven of an impure acarbose;

Step 202, adding an cation exchange resin containing 250 mg sugars/g resin, which the resin is AMBERJET 1200 Na resin (Rohm and Hass Company), into the impure acarbose to get a solution;

Step 204, blending the solution 10 minutes and taking an upper liquid;

Step 206, adding a strong cation exchange resin

containing 80 mg sugars/mL, which the resin is AMBERJET 1200 H resin (Rohm and Hass Company) into the upper liquid to get a mixing solution;

Step 208, mixing and shaking the mixing solution 10 minutes to make the strong cation exchange resin absorbing acarbose;

Step 210, using 1.0N sodium chloride solution to eliminate an impurity in the acarbose; and

Step 212, using ammonia solution to eliminate the rest of an impurity in the acarbose to get a high pure acarbose, which the purity of acarbose is 78%, 1100 mg.

Example 3:

Refer to Fig. 4, the present invention comprises the steps of:

Step 300, adjusting pH value between six and seven of an

upper liquid from an impure acarbose mixing a strong cation exchange resin;

Step 302, passing the upper liquid through a strong cation exchange resin column, 8×50 cm, containing AMBERJET 1200 H resin(Rohm and Hass Company) and washing the strong cation exchange resin in the column by deionized water till the absorbance of strong cation exchange resin being zero or steady;

Step 304, getting an acarbose-containing fractions by using gradient 0.5~1.5N ammonia solution to solve the strong cation exchange resin;

Step 306, concentrating the acarbose to be a volume by a concenteration system; and

Step 308, using alcohol for precipitation the acarbose to get a high pure acarbose, which the purity of the acarbose is up 85%, 920 mg.

Example 4:

Refer to Fig. 5, the purity of the acarbose powder of present invention is 85% from Example 3 and uses in this example comprising the steps of:

Step 402, solving a powder of acarbose, which the purity is 83%~87%, by distilled water to be a solution;

Step 404, adjusting pH value between six and seven of the solution ;

Step 406, passing, flow velocity 1.5mL/min, the solution through α -amyloglucosidase column, 8×30 cm, containing AMBERJET 4400 OH(Rohm and Hass company) and α -amyloglucosidase and washing the α -amyloglucosidase column by using twice times deionized water volume as the α -amyloglucosidase column or the absorbance being 210 nm and steady;

Step 408, eluting an acarbose from the α -amyloglucosidase column by 65℃ distilled water;

Step 410, concentrating the acarbose-containing fractions to be a volume by a concenteration system; and

Step 412, using alcohol for precipitation the impure acarbose to get a high pure acarbose, which the purity of the acarbose is 95%, 900mg.

Advantages of the Invention

The above four examples could get high pure acarbose to be a medical drug, and simplify the processes and use low-cost resin to decrease the product costs.

Therefore, the foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes

will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents may be resorted to, falling within the scope of the invention.